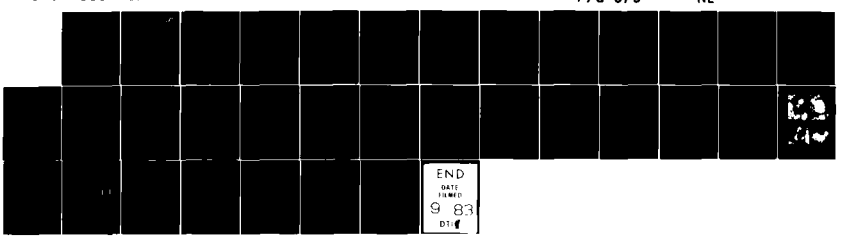
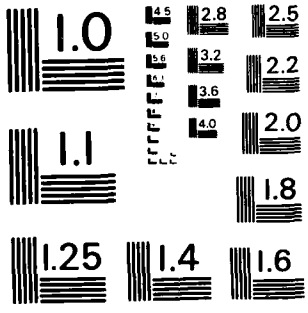
AD-A132 459 KOREAN HEMORRHAGIC FEVER(U) KOREA UNIV SEOUL COLL OF MEDICINE H W LEE 31 MAR 81 DAMD17-80-G-9468 1/1

UNCLASSIFIED F/G 6/5 NL





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS - 1963 - A

AD

AD A132450

KOREAN HEMORRHAGIC FEVER

Final Report

HO WANG LEE, M. D.

March 1981

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Grant No. DAMD 17-80-G-9468

Korea University College of Medicine
Seoul, Korea

DTIC
ELECTE
SEP 15 1983
S D
D

Approved for public release; distribution unlimited

DTIC FILE COPY

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

83 09 14 028

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER DAMD 17-80-G-9468	2. GOVT ACCESSION NO. A132459	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) KOREAN HEMORRHAGIC FEVER		5. TYPE OF REPORT & PERIOD COVERED Final Report 01/01/80 - 12/31/80
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Ho Wang Lee		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-80-G-9468
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Microbiology Korea University College of Medicine Myungyundong, Chongnoku, Seoul 110, Korea		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A.3M161102BS10.AP.053
11. CONTROLLING OFFICE NAME AND ADDRESS Headquarter, US Army Medical Research and Development Command Fort Detrick, Frederick, MD 21701		12. REPORT DATE 03/31/81
		13. NUMBER OF PAGES 33
14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

To be published in the Lancet and J. Trop. Med. and Hyg.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Hantaan virus, Electron microscopy, Cytoplasmic viroplasm, Icosahedral structure, Immunoelectron microscopy, Apodemus agrarius, Horizontal transmission.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The causative agent of Korean hemorrhagic fever was first isolated in 1976 from the rodent Apodemus agrarius coreae and it is registered as Hantaan virus.

This report presents the results on 1) the first

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

visualization of Hantaan virus by EM and 2) intraspecific transmission of the virus in Apodemus mice.

The morphology and morphogenesis of 3 strains of Hantaan virus were examined by thin-section and negative EM of infected A549 cell-culture specimens. Virus was detected within cytoplasmic granular matrices of the infected cells. Virus particles were spherical (diameter 73 ± 5 nm), and had on extremely electron-dense core (diameter 47 ± 6 nm). As infection progressed, viral particles increased in number and were packed into the granular matrices as cytoplasmic crystalline arrays. Negative-contrast staining showed that the virus had an icosahedral structure and annular surface capsomeres. Viruses clumped when exposed to anti-Hantaan virus serum from a convalescent patient. The morphology and morphogenesis of the virus was compatible with those of orbiviruses.

Intraspecific transmission of Hantaan virus in Apodemus mice was determined. The virus excretion in urine, saliva, and feces occurred from about day 10 through day 440 (urine) post-inoculation. Horizontal contact infection occurred among cage-mates up to 440 days after infection.

Accession For
NTIS GRA&I [X]
DTIC TAB [ ]
Unannounced [ ]
Justification
By
Distribution/
Availability Codes
Dist | Avail and/or Special
A

KOREAN HEMORRHAGIC FEVER

Final Report

HO WANG LEE, M. D.

March 1981

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Grant No. DAMD 17-80-G-9468

Korea University College of Medicine
Seoul, Korea

SUMMARY

The morphology and morphogenesis of three isolates of Hantaan virus, the aetiologic agent of Korean haemorrhagic fever (KHF), were examined by thin section and negative contrast electron microscopy of infected A549 cell culture specimens. In thin sections, virus was detected within the cytoplasmic granular matrices of the infected cells. Virus particles appeared to be spherical (diameter 73 $\pm$ 5 nm), with an extremely electron dense core (diameter 47 $\pm$ 6.5 nm) surrounded by a more electron-lucent outer shell. Replication and maturation of the virus appeared to occur in the cytoplasmic viroplasm. As infection progressed, the viral particles increased in number and were packed into the granular matrices as cytoplasmic crystalline arrays. Release of virus from the infected cells seemed to occur by cell dissolution. The virus had an icosahedral structure (diameter 80 $\pm$ 2 nm) with negative contrast staining and had surface doughnut-shaped capsomers and clumped when exposed to anti-Hantaan virus serum collected from a convalescent patient. The morphology and morphogenesis of the virus was compatible with the orbivirus group.

Experimental parameters of infection and intraspecific transmission of Hantaan virus in Apodemus agrarius rodents were determined. Mice inoculated by intramuscular route experienced viremia for about 1 week beginning on day 7. Virus was recovered from lung, kidney, salivary gland, and liver, and

virus excretion in urine, saliva, and feces occurred from about day 10 through day 440 (urine) post-inoculation. Horizontal contact infection occurred among cage-mates up to 440 days after infection.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animals, Resources, National Academy of Sciences-National Research Council.

TABLE OF CONTENTS

Page No.

TITLE i

SUMMARY ii

FOREWORD iii

TABLE OF CONTENTS iv

INTRODUCTION 1

MATERIALS AND METHODS 2

RESULTS 8

Electron microscopic appearance of Hantaan virus. . . 8

Persistence of virus and antibodies in infected *Apodemus* 10

Horizontal transmission of Hantaan virus among *Apodemus* 11

DISCUSSION 12

LITERATURE CITED 15

LIST OF FIGURES AND TABLES

Figure 1. Hantaan virus in A549 cells showing two cytoplasmic granular viral matrices 18

Figure 2. Higher magnification of a viral matrix. . . 18

Figure 3. Hantaan virions in a negative contrast preparation from A549 cells 18

Figure 4. Immunoelectron micrograph of Hantaan virions 18

Figure 5. The course of infection and infectivity of Hantaan virus in *Apodemus agrarius*. 20

Table 1. Quantitative infectivity in excreta and organs of *Apodemus agrarius* infected with Hantaan virus 21

Table 2. Transmission of Hantaan virus from infected *Apodemus agrarius* to normal *Apodemus agrarius* in a cage 22

DISTRIBUTION LIST 23

Page No

LIST OF PUBLICATIONS 25

LIST OF PERSONNEL RECEIVING GRANT SUPPORT 25

INTRODUCTION

Korean haemorrhagic fever (KHF) was recognized for the first time in Korea in 1951 among United Nations personnel during the Korean War (1). The long-sought aetiological agent of KHF was first isolated from the rodent Apodemus agrarius coreae (2,3). Similar diseases to KHF have been recognized in Russia, China, Scandinavia, Eastern Europe and Japan (4-8). There is a close serological realtionship between KHF and the diseases known as nephropathia epidemica in Scandinavia (9,10); haemorrhagic fever with renal syndrome in the Soviet Union (3); epidemic haemorrhagic fever in Japan (11); and epidemic haemorrhagic fever with renal syndrome in China (12,13). The aetiological agent of KHF has been propagated in a human cell culture line (14); it was designated as Hantaan virus and has been so registered in the Catalogue of Arthropod-borne Viruses and Selected Vertebrate Viruses of the World (15). This report describes 1, the first visualization of the Hantaan virus by electron microscopy (EM) and 2, additional data on intraspecific transmission of the Hantaan virus in the rodent Apodemus agrarius which were not included in the previous report.

MATERIALS AND METHODS

I. For EM study of Hantaan virus:

Virus:

Three strains of Hantaan virus, 76/118, Han and Park, were used in the EM experiments. Strain 76/118 was initially isolated from lungs of Apodemus agrarius coreae (3) and the 3rd Apodemus passage was cultivated in A549 cells (see below) and the 11th cell passage was used for EM. The Han strain was isolated from the serum of a 22-year-old male KHF patient. After a single passage in Apodemus, the virus was grwon in A549 cells and the 6th passage was used. Park strain was isolated from the serum of a 31-year-old male KHF patient which had been inoculated directly on A549 cells and the 6th passage was used. The park and Han strains produced cytopathic effects (CPE) after 3 blind cell passages on the 4th day after inoculation whereas strain 76/118 failed to produce CPE up to 2 weeks after inoculation.

Cell culture:

The A549 cell line used was derived from a human pulmonary carcinoma (16). It was obtained from the American Type Culture Collection (CCL-185) and was grown as described previously (14). Monolayers, inoculated with virus stocks passaged in the A549 cells at a concentration of 10^4 Apodemus ID_{50} per 140 cm^2 surface, were harvested on the 4th day (Han and Park strains)

and on the 7th, 11th and 12th days (strain 76/118), after inoculation.

Ultrathin section EM:

The virus infected and control A549 cells were centrifuged into pellets at 700 rpm for 10 minutes, prefixed in 4% buffered glutaraldehyde at 25° C for 24 hours, washed 3 times in phosphate buffer, post-fixed in 1% buffered osmium tetraoxide, dehydrated in acetone and embedded in Epon. They were then sectioned and double stained with uranyl acetate and lead citrate and examined in a Hitachi H-500 electron microscope.

Negative contrast EM:

The A549 cells were harvested 4 days post-inoculation with Han and Park strains after removing the supernatant medium and rinsing once with 0.01M Tris-HCl, pH 8.5. The cells suspended in the buffer were disrupted by 20 strokes in a Ten-Broeck homogenizer, freeze-thawed 5 times, centrifuged at 2,000 rpm for 15 minutes and the supernatant collected. Before EM examination all virus preparations were inactivated by heat at 56° C for 30 minutes, followed by UV light irradiation for 6 minutes, and lyophilized. The lyophilized vials were reconstituted in distilled water and mounted on carbon coated Formvar membrane grids and examined with the electron microscope after staining with 2% potassium phosphotungstate at pH 7.2.

For immunoelectron microscopy (IEM), the grids were preabsorbed with 1:100 dilutions of either KHF-patient serum containing an immunofluorescent antibody titer of 1:4,096 against Hantaan virus, or normal human serum, before mounting. In addition, the virus and control specimens were reacted with either the KHF antiserum or normal serum at 4° C for 48 hours before mounting on the grids.

II. For study of transmission of Hantaan virus in Apodemus agrarius:

Virus strains:

Two strains of Hantaan virus were used in the study of transmission of the virus in Apodemus mice; Strain 76/118 (3) was passaged 8 times in Apodemus agrarius. The ID_{50} for Apodemus of the pool used was $10^{5.9}$/0.3 ml. The Lee strain was recovered from blood of a KHF patient, was passaged 3 times in Apodemus, and contained $10^{8.2}$ ID_{50}/0.3 ml. Both strains were prepared as 10% w/v suspensions of lung tissue, and both were specifically neutralized by sera from a convalescent KHF patient and a rabbit infected with Hantaan virus.

Animals:

Apodemus agrarius of the subspecies Coreae and Jejuensis were trapped on Chin and Jeju islands, respectively, where clinical KHF has not been recognized. More than one-hundred animals of each subspecies were sacrificed and lung tissues

and sera were examined for KHF fluorescent antigen and antibodies. All were negative. Numerous graved animals were collected in spring and fall seasons and offspring of these were raised in the laboratory and used in some of the experiments. Animals employed in these studies weighed 20-40 g. All animals inoculated with virus, with exception of those destined for aerosol intercage transmission studies, were kept in cages covered with polyester filter bonnets (Envirogard^{R}) until sacrificed.

Detection of infection:

Susupensions of tissues, secretions, and ectoparasites were prepared in phosphate buffered saline, pH 7.6 containing 0.2% serum bovine albumin(PBS). Penicillin, streptomycin, and mycostatin in concentrations of 200 u/ml, 200 ug/ml and 100 ug/ml, respectively were added to this diluent and suspensions were clarified by centrifugation at 1,000 g for 20 minutes at 4° C prior to testing. Infectivity titrations were done by intramuscular inoculation of 3-5 Apodemus rodents per 10-fold dilution with 0.3 ml of suspension. Animals were sacrificed between 16 to 20 days later and frozen scetions of lung tissue were examined by immunofluorescence as previously described (3). Specific antigen was taken as evidence of infection and titers were calculated according to Reed and Muench (17). In some cases, tissues of experimentally infected animals were directly

examined for Hantaan virus antigen and its presence was taken as evidence of viral infection, The indirect fluorescent technique (IFT) was employed with FITC-conjugated anti-mouse immunoglobulin (Hyland Laboratories, Costa Mesa, CA).

Animal specimens:

Heparinized blood samples were obtained by cardiac puncture at intervals following experimental infection of Apodemus. Throat swab specimens were expressed into 1 ml of PBS to yield an estimated initial dilution of 1:50. Feces were collected during rodent autopsies to prevent urinary contamination and were homogenized in PBS to make 10% suspensions. Urine samples were collected as follows: Apodemus rodents, either individually or in groups of up to 10 which had been infected at the same time, were placed in rodent metabolic cages. Pears were used as food-water source and urine was collected during an interval of 3-5 hours, in sterile bottles containing 10 ml of Hanks balanced solution(BSS) with 1% bovine serum albumin, pH 7.2. Collection bottles were packed in wet ice. Urine pH was between pH 7.8-8.0 prior to assay for virus.

Experimental design:

1. Route of infection

Susceptible Apodemus rodent were inoculated with 0.3 ml of virus dilutions by intramuscular route.

2. Virus persistence

Apodemus infected by intramuscular route with 1,000 ID_{50} of virus, Lee strain, were autposied at intervals of up to one year in order to ascertain duration of virus infectivity and antigen persistence in various organs and secretions.

3. Virus transmission

Groups of 10 Apodemus were inoculated intramuscularly with 1,000 ID_{50} of virus, Lee strain. Five uninoculated animals were placed in the same large cage with these animals on the day of inoculation. Every 5 days the exposed mice were removed from the infected cage and 5 new animals were introduced. Exposed animals were held in individual cages for a further period of 30 days. These were sacrificed and lung tissues were examined for presence of KHF antigen. This experiment was replicated except that both inoculated and exposed animals were first anesthetized, brushed, and sprayed twice with a 5% suspension of DDT in order to remove or kill all ectoparasites prior to inoculation with, or potential exposure to virus. In addition to these experiments, normal Apodemus mice were exposed to infected Apodemus for 10 to 35 days in the same cage to acquire infection up to 440 days after inoculation of the virus.

RESULT

Electron microscopic appearance of Hantaan virus

A549 cells infected with the three different Hantaan virus isolates, namely 76/118, Park and Han strains, were examined. All three isolates showed identical morphology and morphogenesis.

By thin section EM, viruses were found in the cytoplasm of infected cells in intimate association with granular, electron dense matrices (Fig. 1,2). These matrices, the viroplasms, occurred randomly, varied widely in size and were irregular in shape and unbound. These inclusion bodies were frequently observed in the juxtanuclear positions. As infection progressed, the viral particles increased in number and the proportion of cytoplasmic matrices was reduced. Viral particles were detected either at the edge of matrices or throughout the inclusion and the viruses were packed in the matrices as cytoplasmic crystalline arrays. In the cytoplasm, distended endoplasmic reticulum cisternae were frequently observed. Thread-like or filamentous structures were visible both within the viroplasms and also at more distant sites from the cytoplasmic inclusions. Greater numbers of multilamellar bodies were visible in the nearby sites of the cytoplasmic virus matrices (Fig. 1). The virus particles consisted of a uniformly round, electron-dense core surrounded by a more electron-lucent shell. The diameter of the particles was 73$\pm$ 5 nm with a core diameter of 47 $\pm$ 6.5 nm.

In some particles, electron dense cores were absent and these electron-lucent particles may represent immature virus. Almost all virus particles, whether located intracytoplasmically or extracellularly were unenveloped. Occasionally virus particles were observed in the cavity of the lamellar body and within cytoplasmic vesicles. The release of virus from infected cells seemed to occur as a result of cell dissolution and virus budding was not observed. Viruses were not detected in the nucleus of the infected cells. The virus particles were observed 4 days after infection with Han and Park strains whereas, with strain 76/118, virus was detected at days 11 and 12 post-infection but not at day 7.

Uninfected control cells showed neither cytoplasmic virus matrices nor the virus-like structures. Multilamellar bodies and microfilaments were visible in the cytoplasm of the uninfected cells but in much smaller numbers.

By negative contrast EM, the virus particles appeared to be spherical possessing a double capsid shell. The outside diameters of the outer and inner shells are about 80 nm and 55 nm, respectively; the diameter of the central cavity is about 48 nm (Fig. 3). They had large, ring-shaped surface capsomers, about 14 nm in diameter, with a hollow center approximately 4 nm in diameter. Both intact and many more empty profiles were visible. An envelope was not visible on the virus particle.

By IEM, many more particles were visible when the antiserum absorbed grids were reacted with the virus preparations than in those either preabsorbed with normal serum or not exposed to serum. Aggregated virus particles were also observed and particle surfaces became indistinct when the virus specimens were reacted with KHF antiserum (Fig. 4). However, this aggregation of the viruses did not occur in the virus specimens mixed with normal serum. Uninfected A549 cells extracts never showed virus-like particles.

Persistence of virus, antigen, and antibodies in infected Apodemus

As shown in Table 1 and in Figure 5, viremia was transitory from day 7 to 12 after inoculation. The pattern of virus distribution in kidneys and liver was not as diffuse as in lungs and we found widely scattered small foci of antigen. Virus was first detected in lungs 12 days after inoculation and persisted up to 180 days after infection (Figure 5). Antigen but not infectious virus was present in this organ at least 440 days after infection. Virus was recovered from kidney and parotid glands from about 15 to 43 and 12 to 46 days after infection and antigen was detected up to 60 and 260 days, respectively. The presence of non-infectious virus antigen in these organs is shown by diagonal hatching in Figure 5.

Salivary virus excretion was documented between 9 and 40 days post inoculation. Some specimens contained $10^{3.9}$ ID_{50}/ml.

Large amounts of virus were found in urine ($10^{1.8}$-$10^{4.1}$ ID_{50}/ml) between 9 to at least 489 days after infection. Virus was found in small amounts in feces between 12 and 40 days after infection. Amounts of virus in blood, excreta, and various organs of Apodemus mice are shown in Table 1. High titers of neutralizing (1:1600) and low levels of immunofluorescent antibodies (1:32) to Hantaan virus persisted for over 360 days post inoculation.

Horizontal transmission of Hantaan virus among Apodemus

As shown in Table 2 and Figure 5, Apodemus mice exposed to infected Apodemus for 5 days in the same cage acquired infection begnning 10 days after inoculation of the source cohort, and further groups of animals became infected when caged with Apodemus infected up to 40 days previously. This corresponded well with the period when virus was excreted in saliva, urine, and feces. Results were not different when ectoparasitized and clean animals were used in these experiments. Apodemus mice exposed to infected Apodemus for 20 days in the same cage acquired infection up to 440 days after inoculation of the virus.

DISCUSSION

It is believed that the virus particles observed in this study represent the causative virus of KHF for the following reasons; 1) 3 different isolates of Hantaan virus showed identical morphology and morphogenesis; 2) in blind determinations, specific coded antisera reacted with the virus particles and caused clumping of the viruses; 3) when the virus particles were inoculated into Apodemus mice they developed specific antibodies to Hantaan virus; 4) specific Hantaan virus antigen was produced in the lungs of Apodemus agrarius coreae following inoculation with infected cell cultures and the specific antigen was again detected when these infected lung homogenates were reinoculated in A549 cells on several occasions; 5) the locations of viral antigen detected by immunofluorescent tests were similar to those of the cytoplasmic virus matrices and viral crystalline arrays; 6) the size of the particles detected was compatible with a filtration experiment, in which infectivity was detected from the filtrate of a 100 nm membrane but not from that of a 50 nm membrane (15,18); and 7) virus particles and cytoplasmic granular matrices were never detected in uninfected A549 cells.

Previously "virus-like" structures were observed in the form of crystalline arrays from lungs of Hantaan virus infected but not from normal Apodemus agrarius coreae lungs (19). Re-examination of the virus infected and uninfected Apodemus

lungs resulted in the observation of the same laminated and lattice-like structures in the alveolar lumen of all infected and uninfected lungs examined. It was also noted that these structures were occasionally phagocytized by alveolar microphages. A moderate number of the lattice structures commonly occur in mammalian lungs and have been associated with surfactant discharged from Type 11 alveolar epithelial cells (20-22).

Synthesis and maturation of the virus particles seemed to be occurring in the cytoplasmic viroplasms. This characteristic of viral replication entirely within the cytoplasmic granular matrices, together with the frequent association of filamentous structures with the viral maturation sites, swelling of the endoplasmic reticulum and the spherical structure of the viruses with large, doughnut-shaped capsomers on their surfaces, appeared to be strikingly similar to those of other orbiviruses (22-25). It has been reported that Hantaan virus was labile at low pH and inactivated by heat at 56° C for 30 minutes, characteristics which are shared by orbiviruses (23). However, Hantaan virus was also labile to treatment with ether, chloroform or deoxycholate (15,18). Although most orbiviruses were reported to be relatively resistant to solvents, some, such as the Florio strain of Colorado tick fever virus and strain Eg Ar 1170 of Chenuda virus, were more sensitive to ether, chloroform and sodium deoxycholate (24-27). Lack of any serological relationship between Hantaan virus and some of orbiviruses and other known viruses has been proved (15).

Classification of the Hantaan virus in a taxonomic group requires additional information, such as type and strandedness of nucleic acids. It appears that it contains double stranded RNA. In A549 cell cultures, the virus was resistant to treatment with bromodeoxyuridine (15, 18) indicating that the virus contains RNA. Our preliminary results also showed that the cytoplasmic inclusions in the Hantaan virus infected A549 cells stained orthochromatically green when treated with 0.01% acridine orange in McIlvaine's buffer at pH 3.8 (28) which is indicative of the presence of double stranded nucleic acids in these inclusions (23,29).

Experimental inoculation of adult wild-caught Apodemus agrarius rodents with Hantaan virus resulted in an infection marked by transient viremia, the development of persistent immunofluorescent and neutralizing antibodies, and what might best be termed a semichronic pattern of visceral infection in which significant amounts of virus were excreted in urine, saliva, and to a lesser extent in feces, for periods of up to one year and a half beginning 10-15 days after inoculation.

This type of host response provides excellent experimental evidence confirming the long-held epidemiological suspicion that rodent urine, saliva and feces either directly contaminating food or as asrosolized dust, are the most likely vehicle for transmission of infection to man (30,31,32).

Our data also permit a partial explanation of the mode of intra-specific maintenance of Hantaan virus in Apodemus. We have demonstrated that horizontal infection among individuals occurs 440 days after infection when kept susceptible rodents for 20 days with infected mice in a cage.

LITERATURE CITED

1. Smadel, J. E. Epidemic hemorrhagic fever. Amer. J. Pub. Health, 43: 1327-1330, 1953.

2. Lee, H. W. and Lee, P. W. Korean hemorrhagic fever I. Demonstration of causative antigen and antibodies. Korean J. Intern. Med., 19: 371-383, 1976.

3. Lee, H. W., Lee, P. W. and Johnson, K. M. Isolation of the etiologic agent of Korean hemorrhagic fever. J. Infect. Dis., 137: 298-308, 1978.

4. Smorodintsev, A. A., Alshuller, I. S., Dunaevskii, M. I., Kokhreidze, K. A., Neustroev, V. D. and Churilov, A. A. In etiology and clinical features of hemorrhagic nephroso-nephritis. Smorodintsev, A. A. (ed). Academy of Medical Sciences, Moscow, 26-47, 1944.

5. Ishii, S. Studies on Song-go fever. Jap. Army Med. J., 355: 1755-1758, 1942.

6. Brummer-Korvenkontio, M., Vaheri, A., Hovi, T., von Bonsdorff, C-H., Vuorimies, J., Manni, T., Penttiven, K., Oker-Blom, N. and Laehdevirta, J. Nephropathia epidemica: Detection of antigen in bank voles and serologic diagnosis of human infection. J. Infect. Dis., 141: 131-134, 1980.

7. Gajdusek, D. C. Virus haemorrhagic fever: Special reference to haemorrhagic fever with renal syndrome (epidemic haemorrhagic fever). J. Pediat, 60: 841-857, 1962.

8. Tamura, M. Occurrence of epidemic haemorrhagic fever in Osaka City: First cases found in Japan with characteristic feature of marked proteinuria. Biken J., 7: 79-94, 1964.

9. Svedmyr, A. H., Lee, H. W., Berglund, A., Hoorn, B. Nystrom, K. and Gadjusek, D. C. Epidemic nephropathy in Scandinavia is related to Korean haemorrhagic fever. Lancet, 1: 100, 1979.

10. Lee, H. W., Lee, P.W., Laehdevirta, J. and Brummer-Korvenkontio, M.: Etiological relation between Korean haemorrhagic fever and nephropathia epidemica. Lancet, 1: 186-189, 1979.

11. Lee, H. W., Lee, P. W., Tamura, M., Tamura, T. and Okuno, Y. Etiological relation between Korean haemorrhagic fever and epidemic haemorrhagic fever in Japan. Biken J., 22: 41-45, 1979.

12. Lee, P. W., Gajdusek, D. C., Gibbs, C. J. and Xu, Z. Y. Etiological relation between KHF and epidemic hemorrhagic fever with renal syndrome. Lancet, 1: 819, 1980.

13. Lee, P. W., Gibbs, C. J., Gajdusek, D. C., Hsiang, C. M. and Haiung, G. D. Identification of epidemic haemorrhagic fever with renal syndrome in China with Korean hemorrhagic fever. Lancet, 1: 1025, 1980.

14. French, G. R., Foulke, R. S., Brand, D. A., Eddy, G. A., Lee, H. W. and Lee, P. W. Propagation of the etiologic agent of Korean haemorrhagic fever in a cultured continuous cell line of human origin. Science, 211: 1046, 1981.

15. Information Exchange Subcommittee, American Committee on Arthropod-Borne Viruses, Karabatsos, N. ed. Box 2087, Ft. Collins, C080522.

16. Lieber, M., Smith, B., Szakal, A., Nelson-Rees, W. and Todaro, G. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int. J. Cancer, 17: 62-70, 1976.

17. Reed, L. J. and Muench, H. A simple method of estimating fifty percent end points. Amer. J. Hyg., 27: 493, 1938.

18. Lee, H. W. Final report. Korean hemorrhagic fever. US Army Medical Research and Development Command, Frederick, MD 21701 March 1980.

19. Lee, P. W. and Lee, H. W. Electron microscopic findings of Korean haemorrhagic fever virus. J. Korean Med. Asso., 21: 405-418, 1978.

20. Askin, E. B. and Kuhn, C. The cellular origin of pulmonary surfactant. Lab. Invest., 25: 260-268, 1971.

21. Dermer, G. B. The pulmonary surfactant content of the inclusion bodies found within type II alveolar cells. J. Ultrastructure Res., 33: 306-317, 1970.

22. Rybicka, K., Daly, B. D. T., Migliore, J. J. and Norman, J. C. Ultrastructure of pulmonary alveoli of the calf. Amer. J. Vet. Res., 35: 213-222, 1974.

23. Joklick, W. K. Reproduction of reoviridae. In comprehensive Virology, Fraenkel-Conrat, H and Wagner, R. R. (eds.). Plenum Press NY, 2: 231-334, 1974.

24. Murphy, F. A., Comeman, P, H., Hansen, A. K. and Gray, G. W. Colorado tick fever virus, and electron microscope study. Virol., 35: 28-40, 1968.

25. Murphy, F. A., Borden, E. C., Shope, R. E. and Harrison, A. Physiocochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus - a new taxonomic group. Electron microscopic studies. J. Gen. Biol. Sci., 13: 273-288, 1971.

26. Schnagl, R. D. and Holmes, I. H. A study of Australia arboviruses resembling bluetongue virus. Aust. J. Biol. Sci., 24: 1151-1162, 1971.

27. Borden, E. C., Shope, R. E. and Murphy, F. A. Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus - a new taxonomic group. Physicochemical and serological studies. J. Gen. Virol., 13: 261-271, 1971.

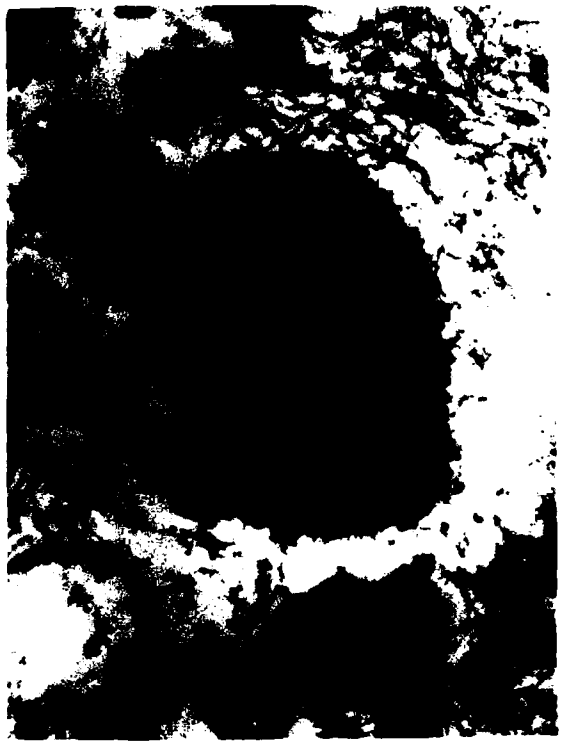
28. Mayor, H. D. Biophysical studies on viruses using the fluorochrome acridine orange. Progr. Med. Virol., 4: 70-86, 1962.

29. Gomatos, P. J., Tamm, I., Dales, S. and Franklin, R. M. Reovirus type 3: Physical characteristics and interaction with L cells. Virol., 27: 441-454, 1962.

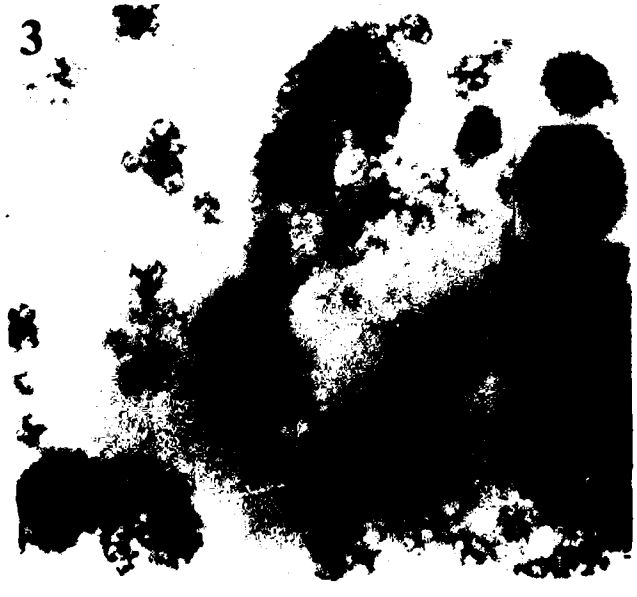
30. Vasyuta, Yu, S. The epidemiology of haemorrhagic fever with renal syndrome in the E.S.F.S.R.Zh. Mikrobiol. Epidemiol. Immunol., 32: 49-56, 1961.

31. Gavrilyuk, B. K. Hemorrhagic nephrosonephritis in Primor'ye Resion. Acad. Med. Nauk. SSR. Ord. Trud. Krasn. Znam. Inst. Eksp. Med., Leningrad, 41, 1968.

32. Lee, H. W. and Johnson, K. M. Human infection by Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the animal room. In preparation.



3



4

FIGURE LEGEND

Fig. 1. Hantaan virus in A549 cells showing two cytoplasmic granular viral matrices located in the juxtanuclear position (x12,000).

Fig. 2. Higher magnification of a viral matrix (x50,000). These matrices varied widely in size and shape and were unbound.

Fig. 3. Hantaan virions in a negative contrast preparation from A549 cells (x100,000). Virus particles are scattered. Inset shows higher magnification of individual virions (x200,000).

Fig. 4. Immunoelectron micrograph of Hantaan virions showing clumping following reaction with a convalescent human serum (x200,000).

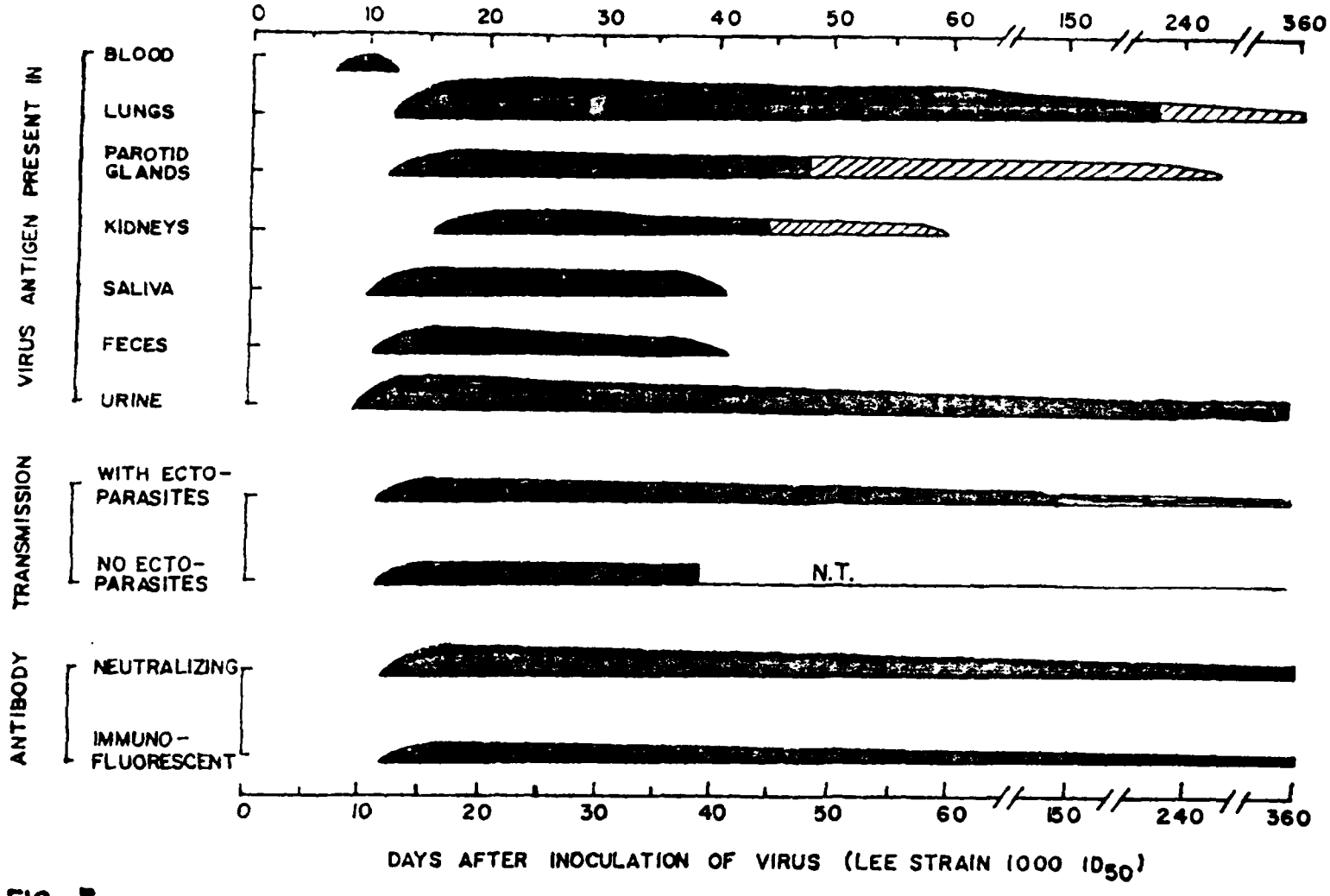


FIG. 5.

THE COURSE OF INFECTION AND INFECTIVITY OF HANTAAN VIRUS IN APODEMUS AGRARIUS

Table 1
Quantitative infectivity in excreta and organs of Apodemus agrarius coreae infected with 1,000 ID_{50} of Hantaan virus (Lee strain AP3)

Excreta & organs	Apodemus -log ID_{50}/ml or g. on days after virus inoculation											
	7	9	12	15	25	35	45	141	181	241	360	489
Blood	1.0	2.4	1.9	<0.5								
Saliva		<2.1	2.6	3.9	3.6	2.2	<2.1					
Urine		1.8	3.8	3.8	4.1	2.2	1.8	2.3	2.1	2.8	1.8	2.8
Feces		<1.5	2.5	3.3	3.0	1.8	<1.5					
Lungs			n.t.	8.7.	n.t.			1.2	<1.2			
Kidneys			n.t.	7.2	n.t.							
Liver			n.t.	6.7	n.t.							
Submaxillary & parotid glands			n.t.	8.2	n.t.							
Lacrimal glands			n.t.	5.6	n.t.							

n.t. : not titrated

Table 2
Transmission of Hantaan virus from infected Apodemus agrarius to normal Apodemus agrarius in a cage

Day post-infection exposed	Virus titer in infected Apodemus (-log/ml) urine	saliva	feces	Days exposed	No. infected/ no. exposed
5				5	0/5
7				5	0/4
10	1.8	< 2.1	< 1.5	5	4/5
15	3.8	3.9	3.3	5	5/5
20				5	4/5
25	4.1	3.6	3.0	5	4/4
30				5	4/5
35	2.2	2.2	1.8	5	3/5
40				5	0/5
45	1.8	< 2.1	< 1.5	5	0/5
55				5	0/5
60				5	0/5
85	2.8	< 2.1	< 1.5	5	0/5
100				5	0/5
119				20	2/5
141	2.3			10	0/4
181	2.1			10	0/4
				20	1/4
212	1.8			10	0/4
				20	0/4
				30	0/4
277	2.8			35	0/3
360	1.8			20	1/4
440	1.8			20	1/4
				40	1/3

DISTRIBUTION LIST

Final Report

4 Copies	Commander US Army Medical Research and Development Command ATTN: SGRD-SI Fort Detrick, Frederick, MD 21701
12 Copies	Administrator Defense Technical Information Center ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1 Copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houstion, TX 78234
1 Copy	Dean, School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 Copy	COL Gerald A. Eddy, DVM US Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD 21701
1 Copy	Dr. William F. Scherer Department of Microbiology Cornell University Medical College 1300 York Avenue New York, N.Y. 10021
1 Copy	COL Philip K. Russell, MC Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D.C. 20012
1 Copy	Dr. Karl M. Johnson US Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD 21701
1 Copy	Dr. Joel M. Darlymple US Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD 21701

Final Report

1 Copy	COL John D. Marshall, Jr., 192-18-7953 Department of Dermatology Research Letterman Army Institute of Research Presidio of San Francisco, CA 94129
2 Copies	Dean Korea University College of Medicine Seoul, Korea
1 Copy	Dr. R. E. Shope Jr. Yale University School of Medicine Yale Arbovirus Research Unit 60 College Street New Haven, Connecticut 06510
1 Copy	Dr. Jordi Casals Yale University School of Medicine Yale Arbovirus Research Unit 60 College Street New Haven, Connecticut 06510
1 Copy	Commander US Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD 21701
1 Copy	Dr. George R. French The Salk Institute Government Service Division P.O. Box 250 Swiftwater, Penn. 18370
1 Copy	Dr. D. C. Gajdusek National Institute of Neurological and Communicative Diseases and Stroke Bethesda, Maryland 20014
1 Copy	Dr. Clarence J. Gibbs, Jr. National Institute of Neurological and Communicative Diseases and Stroke Bethesda, Maryland 20014

LIST OF PUBLICATIONS

1. Lee, H. W., Lee, M. C. and Cho, B. H. Management of Korean haemorrhagic fever. Medical Progress, 15-21 Sep. 1980.

2. French, G. R., Foulke, R. S., Brand , O. A. Eddy, G. A., Lee, H. W. and Lee, P. W. Korean hemorrhagic fever; Propagation of the etiologic agent in a cell line of human origin. Science, 211: 1046-1048, 1981.

LIST OF PERSONNEL RECEIVING GRANT SUPPORT

1. Seong In Wha M.D. Ph.D. Virologist, Fluorescent and Immune Electron Microscopic work.

2. Liu, Sun Ja. Tissue Culture Technician.

3. Kim Soo Am. Field Worker.

4. Park Chong Keun. Driver and Field Worker.

5. Lee, Ji Hyun. Animal Caretaker.

DATE
ILME